62305 82791

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Delia Radulescu
Serial No.:	10/643,913
Filed:	August 20, 2003
Group:	2853
For:	Method for Forming Polymer Microspheres

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INFORMATION DISCLOSURE STATEMENT

Pursuant to Applicant's duty to disclose all relevant material, the Applicant attaches hereto PTO Forms SB/08A and SB/08B "Information Disclosure Statement by Applicant". A copy of the non-patent literature documents cited on Form SB/08B is also enclosed.

Respectfully submitted,

LOCKE LIDDELL & SAPP LLP
Attorneys for Applicant

Martin Korn
Registration No. 28,317

Date: November 19, 2003

2200 Ross Avenue, Suite 2200
Dallas, TX 75201-6776
214/740-8549
214/740-8800 (Fax)

PTO/SB/08a (08-03)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	10/643,913
Filing Date	08/20/2003
First Named Inventor	Delia Radulescu
Art Unit	2853
Examiner Name	
Attorney Docket Number	62305 82791

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	US- 4,138,383	02/06/1979	Rembaum et al.	
	2	US- 4,444,961	04/24/1984	Timm	
	3	US- 4,956,128	09/11/1990	Hommel et al.	
	4	US- 4,981,625	01/01/1991	Rhim et al.	
	5	US- 5,053,100	10/01/1991	Hayes et al.	
	6	US- 5,260,002	11/09/1993	Wang	
	7	US- 5,376,347	12/27/1994	Ipponmatsu et al.	
	8	US- 5,643,506	07/01/1997	Rourke	
	9	US- 5,736,074	04/07/1998	Hayes et al.	
	10	US- 6,224,794 B1	05/01/2001	Amsden et al.	
	11	US- 6,277,413 B1	08/21/2001	Sankaram	
	12	US- 6,331,317 B1	12/18/2001	Lyons et al.	
	13	US- 6,367,925 B1	04/09/2002	Chen et al.	
		US-			
		US-			
		US-			
		US-			
		US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]–Number[4]–Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/08b (08-03)
Approved for use through 06/30/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449B/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	10/643,913
Filing Date	08/20/2003
First Named Inventor	Delia Radulescu
Art Unit	2853
Examiner Name	
Attorney Docket Number	62305 82791

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	See Attachm I		
	2	K. J. PEKAREK, M. J. DYRUD, K. FERRER, Y. S. JONG, E. MATHIOWITZ, "In Vitro and in Vivo Degradation of Double-Walled Polymer Microspheres," Journal of Controlled Release, No. 40, p. 169-178, (1996).	
	3	C. BERKLAND, K. KIM, D. W. PACK, "Fabrication of PLG Microspheres with Precisely Controlled and Monodisperse Size Distributions," Journal of Controlled Release, No. 73, p. 59-74, (2001).	
	4	DANESI, INNOCENTI, FOGLI, GENNARI, BALDINI, DIPAOLO, SALVADORI, BOCCI, CONTE, DELTACCA, "Pharmacokinetics and Pharmacodynamics of Combination Chemotherapy with Paclitaxel and Epirubicin in Breast Cancer Patients," Journal of Clinical Pharmacol, No. 53, p. 508-518, (2002).	
	5	SOUSA-ESCANDON, VAZQUEZ, QUINTERO-ALDANA, PICALLO, NEIRA, GARCIA-NOVIO, MATE0, RICO, MEL, "Neo-Adjuvant Treatment of Infiltrating Transitional-Cell Carcinoma of the Bladder with Paclitaxel and Cisplatin: A Phase II Trial," International Journal of Urology, No. 9, p. 162-166, (2002).	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Information Disclosure Statement by Applicant (PTO/SB/08B); Additional Other Prior Art - Non Patent Literature Documents

Attachment 1

a. 1

b. N. LEELARASAMEE, S. A. HOWARD, C. J. MALANGA AND J. K. H. MA, "A Method for the Preparation of Polylactic Acid Microcapsules of Controlled Particle Size and Drug Loading," J. Microencapsulation, Vol. 5 (No. 2), p. 147-157, (1987).

c.

J. MICROENCAPSULATION, 1988, VOL. 5, NO. 2, 147–157

A method for the preparation of polylactic acid microcapsules of controlled particle size and drug loading

N. LEELARASAMEE, S. A. HOWARD†, C. J. MALANGA and J. K. H. MA‡

School of Pharmacy, West Virginia University, Morgantown, WV 26506, U.S.A.

(*Received 3 June 1987; accepted 30 June 1987*)

A solvent partition technique for the microencapsulation of hydrocortisone–polylactic acid has been developed for the preparation of microcapsules of controlled particle size distribution and drug loading. The method involves continuous injection of a drug–polymer solution with a syringe infusion pump into flowing mineral oil where microcapsules are formed as the solvent of the drug–polymer is partitioned into the mineral oil. Using preselected syringe needle size and mechanical control of the mineral oil flow rate at the needle tip, microcapsules of consistent particle sizes and desired drug loadings were prepared. Microcapsules of different internal structures were also prepared by varying the solvent system for the drug–polymer preparation. Dissolution studies showed that at the same drug loading, the rate of the percentage drug release increased with decreasing particle size, and that at similar particle size distributions, the rate increased with increasing drug loading. These results indicate that both the particle size distribution, and the drug loading must be controlled in a microencapsulation process to produce microcapsules of controlled drug release rate.

Introduction

In recent drug formulation development, increased attention has been given to polymeric drug dosage forms designed for targeted drug delivery and controlled drug release. Microencapsulation, by which a therapeutic agent is incorporated into a micromatrix, preferably a bioerodable polymer, remains a viable approach for the development of a parenteral dosage form. Among the bioerodable polymers, human serum albumin and poly-D,L-lactic acid have been widely tested for the encapsulation of a number of therapeutic agents such as steroids (Beck *et al.* 1983, Benoit *et al.* 1986, Cavalier *et al.* 1986), and anticancer drugs (Sugibayashi *et al.* 1979, Fujimoto *et al.* 1985 a, Spenlehauer *et al.* 1986, Tsai *et al.* 1986). Microcapsules made from either albumin or polylactic acid have been used in several clinical studies (Fujimoto *et al.* 1985 a, Ziyun and Ruiqui 1985).

A drawback for the albumin microcapsules is that the microencapsulation process requires either the denaturation of the protein by heating up to 170°C or the use of a crosslinking agent such as glutaraldehyde to stabilize the microcapsules (Fujimoto *et al.* 1985 a). Studies have shown that both treatments can lead to drug degradation, especially for anticancer drugs such as adriamycin (Cumming and Willmott 1985) and mitomycin C (Mehta *et al.*, private communication 1987). On

† Present address: Ortho Pharmaceutical Corporation, Research Laboratories, Raritan, NJ 08869, U.S.A.

‡ To whom correspondence should be addressed.

the other hand, polylactic acid, a nontoxic polymer of low crystallinity, may be used to prepare microcapsules where problems concerning drug degradation can be avoided. Since polylactic acid undergoes slow hydrolytic degradation with reported *in vivo* half life in the range of 6–12 months (Makino *et al.* 1986), stable microcapsules can be prepared with this polymer for sustained drug release action.

Microencapsulation of drug-loaded polylactic acid has been carried out primarily using oil-in-water emulsion methods (Beck *et al.* 1983, Benoit *et al.* 1986, Cavalier *et al.* 1986). The oil phase consists of drug and polymer in an organic solvent such as methylene chloride while the water phase contains a suitable emulsifier such as polyvinyl alcohol. The emulsion is first prepared by using either a mechanical stirrer or ultrasonic vibrator. Formation of microcapsules is then achieved through solvent evaporation under reduced pressure.

Although the procedure is simple, the consistency of the microcapsules in drug loading and particle size distribution requires careful control of several parameters including drug solubility in the aqueous phase (Juni *et al.* 1985, Chang *et al.* 1986, Spenlehauer *et al.* 1986), oil phase viscosity (Spenlehauer *et al.* 1986), concentration of emulsifying agents and the stirring rate (Benita *et al.* 1984, Chang *et al.* 1986, Spenlehauer *et al.* 1986). For example, an increase in stirring speed and the concentration of the emulsifying agent will produce smaller size microcapsules. Furthermore, there is a common observation in those studies that an increase in drug–polymer content results in larger microcapsule size accompanied by increased drug loading. Thus, it is difficult to prepare microcapsules with different drug loading having the same particle size distribution using the emulsion method. Since both the size and the drug loading have been implicated as the principal parameters for the control of drug release from the microcapsules (Higuchi 1963, Chang *et al.* 1986), we believe that a non-emulsion method in which the size and drug loading of the microcapsules can be independently controlled will be of value. The present study reports a solvent partition method for the preparation of polylactic acid microcapsules and the effects of particle size and drug loading on the rate of drug release from the microcapsules containing hydrocortisone as a model drug.

Experimental

Preparation of hydrocortisone–polylactic acid microcapsules

Poly-D,L-lactic acid (mol. wt 33000; m.p. 150°C) was purchased from Polysciences, Inc., Warrington, Pennsylvania, U.S.A. Micronized hydrocortisone was obtained from the Upjohn Company, Kalamazoo, Michigan, U.S.A. Light mineral oil N.F., methylene chloride and n-heptane were purchased from Fisher Scientific, An Allied Company, Fairlawn, New Jersey, U.S.A. Dimethyl isosorbide was obtained from ICI Americas Inc., Wilmington, Delaware, U.S.A. The microencapsulation of hydrocortisone with polylactic acid was achieved based on a solvent partition method using methylene chloride and mineral oil. The drug–polymer mixture in methylene chloride or in a mixed solvent system containing methylene chloride, ethanol, and water was injected to form tiny droplets into the flowing mineral oil. Since mineral oil is miscible with methylene chloride but does not dissolve the drug or the polymer, formation of drug-polymer microspheres occurred as the methylene chloride was extracted into the mineral oil. The above process was carried out using an apparatus designed in this laboratory, a diagram of which is

low crystallinity, may be used
ing drug degradation can be
tic degradation with reported
Makino *et al.* 1986), stable
sustained drug release action
has been carried out primarily
J, Benoit *et al.* 1986, Cavalier
r in an organic solvent such as
a suitable emulsifier such as
sing either a mechanical stirrer
then achieved through solvent

of the microcapsules in drug
control of several parameters
et al. 1985, Chang *et al.* 1986,
ucr *et al.* 1986), concentration
t al. 1984, Chang *et al.* 1986,
se in stirring speed and the
e smaller size microcapsules.
se studies that an increase in
size accompanied by increased
ocapsules with different drug
ig the emulsion method. Since
ed as the principal parameters
s (Higuchi 1963, Chang *et al.*
ch the size and drug loading of
will be of value. The present
preparation of polylactic acid
ig loading on the rate of drug
tisone as a model drug.

osules

C) was purchased from Poly-
licronized hydrocortisone was
ichigan, U.S.A. Light mineral
chased from Fisher Scientific,
A. Dimethyl isosorbide was
aware, U.S.A. The microen-
s achieved based on a solvent
neral oil. The drug–polymer
system containing methylene
iny droplets into the flowing
hylene chloride but does not
olymer microspheres occurred
ral oil. The above process was
ratory, a diagram of which is

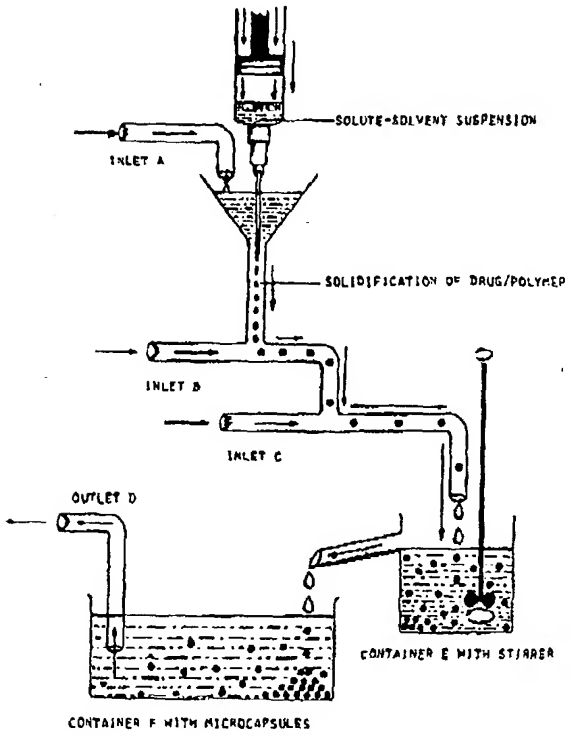


Figure 1. A diagram for the microencapsulation process via solvent partition. The inlets and outlet indicate the circulation of mineral oil flow.

shown in figure 1. This system provides a continuous flow of mineral oil via inlets A, B and C. The flow rate can be adjusted to control the particle size of the microcapsule formation and facilitate the separation of individual microcapsules during the hardening process.

For the preparation, a desired amount of drug was added into a 2 per cent w/w polymer solution in methylene chloride or in a mixed solvent system containing methylene chloride–ethanol–water (95·6 : 4·0 : 0·4) or methylene chloride–ethanol–dimethyl isosorbide (82·0 : 1·6 : 16·4). The drug–polymer mixture was then injected into the mineral oil stream at a constant rate (3·6 ml/h, Harvard Apparatus syringe pump, model 2681, Millis, Massachusetts, U.S.A.) using a selected hypodermic needle size (Syringe pipeting type D, Unimetrics, Anaheim, California, U.S.A.). The downward flow of mineral oil at the needle tip (see figure 1), which dictates, in part, the droplet size, was maintained at a constant rate through the flow rate control of both inlets A and B. The formation and hardening of the microcapsules were completed at container E. The apparatus, in addition to the design shown in figure 1, also contained an automatic mineral oil recycling system which was composed of : (1)

a filter unit to separate the hardened microcapsules from the mineral oil; (2) a heating unit to remove methylene chloride from the mineral oil under reduced pressure; (3) a tank to store the heated mineral oil with the cooling unit; and (4) a tubing pump (Masterflex model 7016, Cole-Palmer Instrument Company, Chicago, Illinois, U.S.A.) to re-circulate the mineral oil back to the apparatus system.

The particle size of the microcapsules was controlled by both the needle size (G) and the flow rate of mineral oil at the needle tip, whereas the drug loading was predetermined by the drug and the polymer content. For the present study, microcapsules of three particle size distributions containing the same drug loading were prepared in triplicate for reproducibility. Microcapsules of similar particle size distribution but with different drug loading were also prepared. The freshly prepared microcapsules were washed twice with heptane and then kept in heptane overnight to remove any trace of organic solvents before allowing to dry. Then they were further washed with distilled water for 15 minutes and dried *in vacuo*. All prepared microcapsules were stored in closed containers in a vacuum desiccator.

Determination of drug loading

The drug loading, which was expressed in weight of drug per total weight of microcapsules, was determined in triplicate for each batch of microcapsules using a uv diode array spectrophotometer (HP-8450A, Hewlett Packard, Palo Alto, California, U.S.A.) at 247 nm. The samples were analyzed as follows. Microcapsules, 10 mg in weight, from each batch were dissolved in 10 ml methylene chloride. After complete dissolution, 40 ml distilled water was added and the mixture was then stirred in a water bath maintained at 50°C. After complete evaporation of methylene chloride, the temperature was further raised to 75°C for 5 min. The solution was then filtered and diluted to 100 ml in a volumetric flask for measurement. The standards were prepared using known amounts of drug and blank microcapsules in the same manner.

Micrographs and the determination of the particle size distribution

Photographs of the microcapsules were taken using a research microscope (Nikon, Japan) equipped with a 35 mm camera (Nikon, Japan). For particle size distribution analysis, a stereomicroscope equipped with an eyepiece scale (Nikon, Japan) was used and the scale was calibrated at 40 × magnification (25 μm per scale unit). The samples were prepared by suspending 10 mg microcapsules from each batch in 10 ml mineral oil. To count and measure the size of the microcapsules, a plexiglass plate marked by rows and columns (10 × 10 blocks, 25 cm^2) was used as the platform over which a 1 ml sample of the suspension was spread to form a uniform single-layer of microcapsules. Individual microcapsules in each block were then counted and measured for size. Although the microscopic observation indicates that microcapsules are spherical in shape, a spheroidal shape is sometimes seen. In this case, the size measurement was all carried out by taking the average between the longest and shortest values of the diameter. With the aid of the computers, the particle size distribution was determined and expressed in mean diameter (μm) $\pm$ standard deviation. The same data were also used to estimate the microcapsule densities. Individual microcapsule volume was determined from the measured size (diameter). With the aid of a computer, the total volume of the microscope samples of known weight and thus the density, were then determined.

om the mineral oil; (2) a heating
oil under reduced pressure; (3) a
ig unit; and (4) a tubing pump
t Company, Chicago, Illinois,
apparatus system.
illed by both the needle size (G)
whereas the drug loading was
nrent. For the present study,
ntaining the same drug loading
ocapsules of similar particle size
e also prepared. The freshly
ptane and then kept in heptane
fore allowing to dry. Then they
inutes and dried *in vacuo*. All
ainers in a vacuum desiccator.

ght of drug per total weight of
batch of microcapsules using a
Hewlett Packard, Palo Alto,
analyzed as follows. Micro-
dissolved in 10 ml methylene
led water was added and the
ained at 50°C. After complete
was further raised to 75°C for
100 ml in a volumetric flask for
nown amounts of drug and blank

ze distribution
using a research microscope
Nikon, Japan). For particle size
with an eyepiece scale (Nikon,
magnification (25 μm per scale
10 mg microcapsules from each
the size of the microcapsules, a
0 blocks, 25 cm^2) was used as the
n was spread to form a uniform
psules in each block were then
scopic observation indicates that
shape is sometimes seen. In this
taking the average between the
the aid of the computers, the
ressed in mean diameter (μm)
ed to estimate the microcapsule
ermined from the measured size
me of the microscope samples of
ermined.

Drug dissolution studies

A dissolution bottle with a teflon-coated screw cap was filled with 20 ml pH 7·4 phosphate buffer and rotated at 39 r.p.m. (model SA7-2424, Ernest D. Menold, Lester, Pennsylvania, U.S.A.) in a water bath of constant temperature at 37°C. After the solution reached equilibrium temperature, microcapsules, weight equivalent to 3 mg hydrocortisone, were added into the bottle. Then the dissolution samples, 4 ml in size, were collected at the appropriate time intervals. Collection of the samples was made using a disposable syringe equipped with 5 μm filtered needle. Sample replacement was made after each collection with the same volume of the dissolution medium maintained at 37°C. The frequency of sampling was predetermined to prevent the drug concentration in the medium from exceeding 10 per cent of its solubility and thus, maintain a perfect sink condition. The hydrocortisone concentrations of the collected samples were determined spectrophotometrically at 247 nm.

Results and discussion

The microencapsulation technique reported in this study is based on a solvent partitioning phenomenon where, during the process, the solvent used for the drug–polymer mixture is being extracted by another solvent which has no solubility for the drug and polymer. This method allows one to prepare microcapsules of two types of drug distribution by proper selection of the solvent systems. A matrix type of microcapsule, in which the drug particles are randomly distributed in the polymer, can be obtained by using a single solvent system, whereas a reservoir type of microcapsule, in which the drug particles reside primarily at the centre core, may be prepared by using a mixed solvent system.

This phenomenon is demonstrated in figure 2 via microscopic examinations of the polylactic acid–hydrocortisone microcapsules prepared using methylene chloride as a single solvent and mixed solvent systems containing methylene chloride as the principal solvent for the polymer. Figure 2 (A) shows microcapsules obtained using methylene chloride as the single solvent. Figure 2 (B) and (C) shows the microcapsules obtained from mixed solvent systems containing methylene chloride–ethanol–water and methylene chloride–ethanol–dimethyl isosorbide, respectively. The dark areas represent drug particles. Here, it is seen that figure 2 (A) shows a matrix type of drug distribution, whereas figure 2 (B) and (C) shows microcapsules which exhibit a centre core with the deposit of drug crystals at and inside the centre core. The reservoir structure is particularly clear for the microcapsules prepared in the mixed solvent system containing dimethyl isosorbide, a solvent which has high solubility for hydrocortisone and is water miscible. The formation of the reservoir type of drug distribution is achieved by the fact that the ethanol–water or ethanol–dimenthyl isosorbide mixture is not miscible with mineral oil but has greater solubility for hydrocortisone. During the microencapsulation process, methylene chloride, the principle solvent for polylactic acid, is partitioned into mineral oil causing the solidification of the polymer around the droplet of the drug solution. Upon drying, drug particles are forced to stay at the inner core of the microcapsules. The drying process may also cause deposit of drug particles at or near the inner side of the polymer wall. This is readily seen in microcapsules prepared from a solvent mixture of methylene chloride, ethanol, and water. As shown in figure 2 (B), the microcapsules are characterized by the presence of a centre ring with deposits of drug particles.

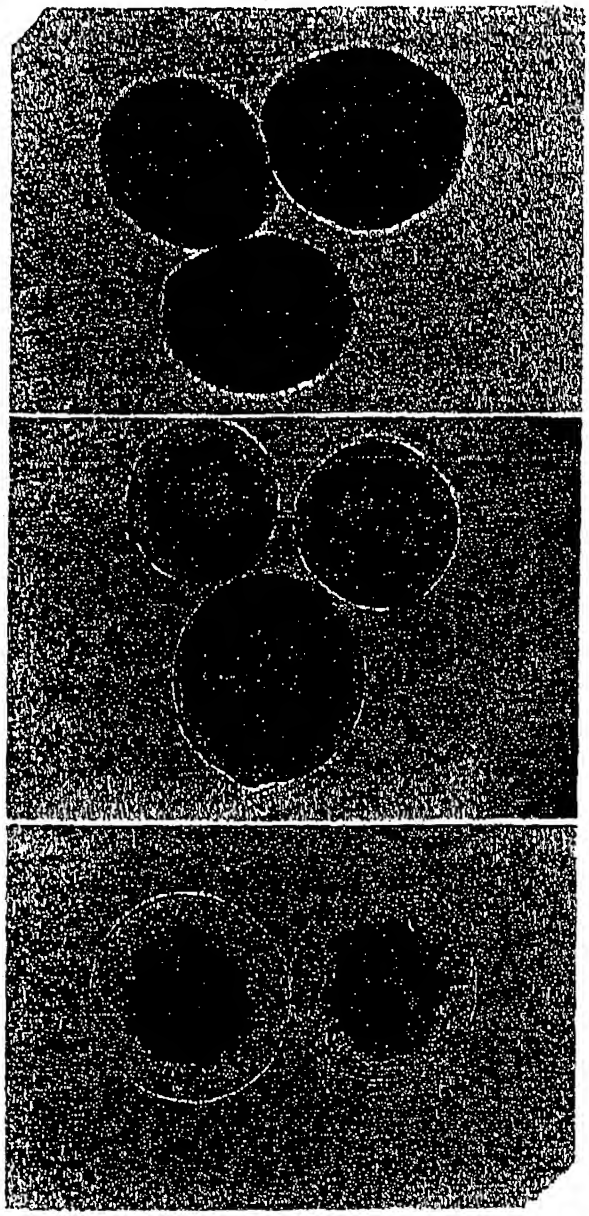
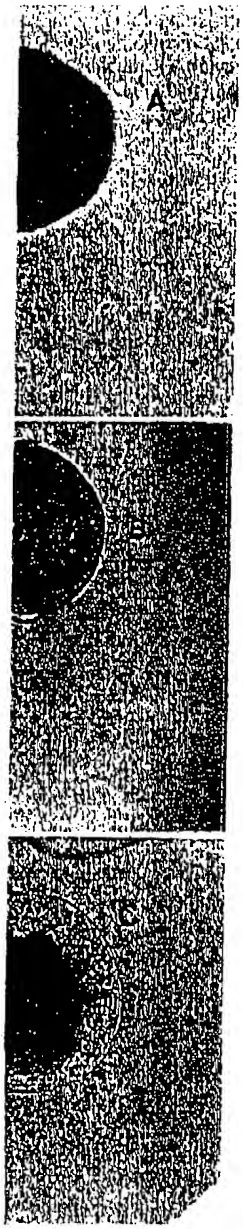


Figure 2. Photographs (100 ×) of microcapsules (average diameter 208 μm) prepared using various solvent systems: (A) methylene chloride, 13·9 per cent drug loading; (B) methylene chloride-ethanol-water, 12·0 per cent drug loading; (C) methylene chloride-ethanol-dimethyl isosorbide, 14 per cent drug loading.



·nge diameter 208 μm) prepared using
de. 13·9 per cent drug loading; (B)
drug loading; (C) methylene chloride-
oading.

The reservoir type of microcapsule was thought to be advantageous because a theoretical model (Crank 1956) has predicted that such a system may exhibit zero-order drug release. However, our study showed that the release curves for the reservoir type of microcapsule were similar to those of the matrix type, presumably due to the porous nature of the polylactic acid microcapsules. Further studies of the reservoir type of microcapsule are currently underway. The present study includes only the results obtained for the matrix type of microcapsule.

Table 1 shows the composition and yield of the microcapsules prepared in triplicate at various particle size distributions. The overall yields of the microencapsulation are well above 90 per cent and the incorporation of the drug into the microcapsules is about 70 per cent. The drug loss is accounted for by the extensive washing procedures used to obtain the final product. The loading, however, is remarkably constant for all the preparations.

Table 2 shows the particle size analysis and reproducibility via the control of mineral oil flow rate and the needle size for the microcapsules prepared at similar drug loading. The results show that reproducible particle size distributions of the microcapsules may be achieved by selected needle size and the mineral oil flow rate at the needle tip. While larger needle size produces larger particles, an increase in the oil flow rate reduces the particle size. By controlling these factors, microcapsules with diameters averaging 145, 221 and 390 μm were prepared. In each particle size distribution, the measured number of particles per 10 mg of microcapsules and the microcapsule density are within 9·0 per cent in variation among the three repeated preparations.

Figure 3 shows the effect of particle size on the rate of drug release from the polylactic acid–hydrocortisone microcapsules. Good reproducibility of drug release curves was obtained from microcapsules of the same size distribution. The results show that microcapsules of the smaller size exhibit a faster rate of drug release than those of the larger size. This is expected since the total surface area of microcapsules is much higher for small particle size than for large particle size at a given weight. For the microcapsules having an average diameter of 145 μm, the amount of drug release was found to reach 90 per cent of the total drug loading within 20 h. For the larger

Table 1. Drug-polymer composition and yield of polylactic acid–hydrocortisone microcapsules.

Lot number	Polylactic acid (g)	Hydrocortisone (g)	Drug loading		Yield (per cent w/w)
			Calculated (per cent w/w)	Observed (per cent w/w)	
S1	0·202	0·050	19·9	13·0	92·7
S2	0·201	0·050	20·1	13·2	92·3
S3	0·201	0·050	20·1	12·4	92·1
M1	0·201	0·044	18·0	13·9	—
M2	0·201	0·044	18·0	13·4	95·7
M3	0·201	0·044	18·0	13·4	94·0
L1	0·201	0·044	18·0	13·2	92·5
L2	0·201	0·044	18·0	12·7	—
L3	0·200	0·044	18·1	12·4	—

Table 2. Analysis of particle size distribution of polylactic acid–hydrocortisone microcapsules.

Lot number	Flow rate (ml/min)	Needle (G)†	Particle size (mean ± s.d.) (μm)	Microcapsules Number (/10 mg)	Microcapsules Density (g/ml)
S1	14·5	25	144·3 ± 60·6	11020	0·365
S2	14·5	25	144·9 ± 43·2	14363	0·338
S3	14·5	25	146·0 ± 43·7	14606	0·342
M1	9·0	25	222·6 ± 62·0	3470	0·403
M2	9·0	25	218·2 ± 53·6	4500	0·343
M3	9·0	25	221·4 ± 53·0	5104	0·293
L1	9·0	22S	412·4 ± 81·2	982	0·249
L2	9·0	22S	373·1 ± 58·2	1157	0·297
L3	9·0	22S	385·5 ± 54·6	1140	0·276

† G = gauge of a needle measured by the outside diameter (cross-section) of the needle shaft (Standard Stubb's English wire gauge: 25 = 510 μm and 22S = 711 μm.

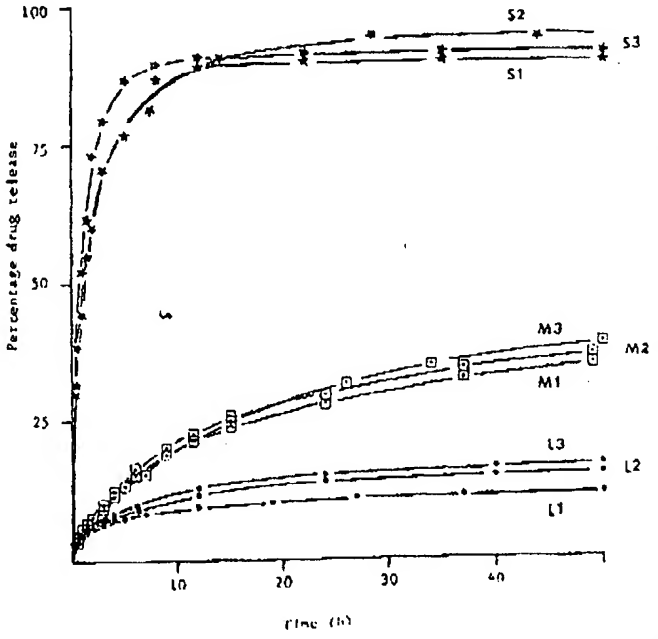
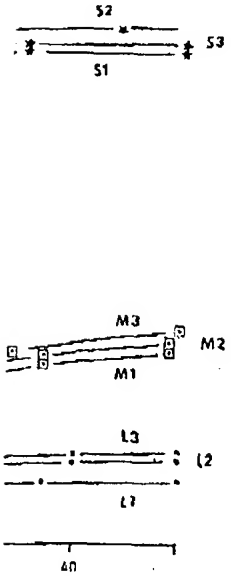


Figure 3. Drug release profiles for the hydrocortisone–polylactic acid microcapsules of various particle size distributions. The loading and particle size distribution of the microcapsules are given in tables 1 and 2.

f polylactic acid–hydrocortisone

e size ±s.d.) n)	Microcapsules Number (10 mg)	Density (g/ml)
±60·6	11020	0·365
±43·2	14363	0·338
±43·7	14606	0·342
±62·0	3470	0·403
±53·6	4500	0·343
±53·0	5104	0·293
±81·2	982	0·249
±58·2	1157	0·297
±54·6	1140	0·276

meter (cross-section) of the needle
1 and 22S = 711 μm.



e–polylactic acid microcapsules of
nd particle size distribution of the

microcapsules with diameters averaging 221 and 390 μm, the cumulative drug release for 100 h was found to be about 45 per cent and 15 per cent respectively. A similar particle size effect for the polylactic acid microcapsules has been reported for other drug systems (Suzuki and Price 1985). The results of the present study, which suggest an exponential increase in drug release with decreasing particle size, are consistent with the prediction of a theoretical model developed by Higuchi (Higuchi 1963) which has been used to describe the diffusional based drug release from polymeric matrices. For a spherical matrix, the drug release kinetics may be expressed by the following equation:

$$1{\cdot}5(1-(1-M_t/M_\infty)^{2/3})-M_t/M_\infty=Bt; \quad B=3C_SD/r_0^2A \qquad (1)$$

where M_t and M_∞ are the amounts of drug released at time t and infinite time respectively, and B is a constant which describes a combined effect of drug solubility in the release medium (C_S), drug diffusivity (D), radius of the matrix (r_0), and the drug loading per unit volume of the matrix (A) on the rate of drug release. It can be seen that the rate constant B is proportional to $1/r_0^2$.

The release curves shown in figure 3 were found to give a biphasic linear relationship according to eqn (1) which corresponds to a fast first stage and a slow second stage drug release from the microcapsules. From the linear plots, the values of B were determined for quantitative comparison of the rate of drug release from the microcapsules of different particle size distributions. Figure 4 shows the plot of log B versus log r_0 for the particle size effect. Although the data are insufficient to verify the relationship that B is proportional $1/r_0^2$, figure 4 clearly indicates an exponential increase in rate of drug release with decreasing particle size. The curvature plot shows that at smaller particle sizes, the rate of drug release is faster than can be predicted by eqn (1). It should be emphasized that eqn (1) is ideal for systems where

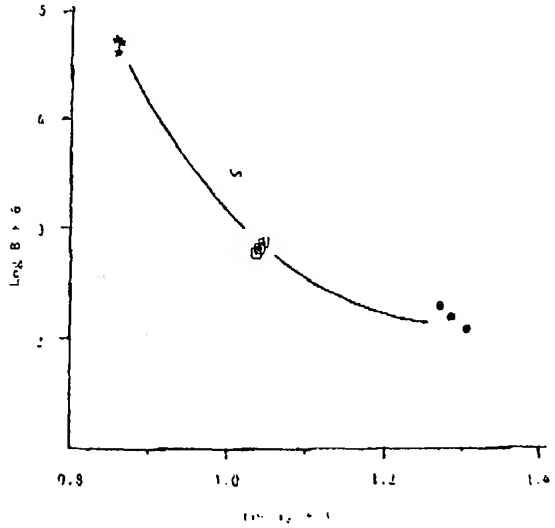


Figure 4. *Effect of microcapsule particle size (r_0 is the average radius) on the rate constant B calculated from eqn. (1) for the first stage drug release.*

drug particles are relatively small in relation to the diffusional distance of the polymeric matrix. Microcapsules containing large drug particles or clusters of drug particles should exhibit an increased rate of drug release. This may be especially true for microcapsules of reduced sizes. Thus, in the microcapsule system, the particle size is perhaps not an independent variable, and that a change in particle size may also change the diffusivity of the system.

The present study shows that the release of hydrocortisone from the polylactic acid microcapsules is via the diffusion of the small molecule through the polymeric matrix. For an non-diffusional system as the ethylene-vinyl acetate copolymer microcapsules of bovine serum albumin, the release of the macromolecules have been shown to increase with increasing particle size (Siegel and Langer 1984).

Figure 5 shows the rate of hydrocortisone release from microcapsules of similar particle size distribution but of different drug loading. The results show that the rate of percentage drug release increases with increasing drug loading. Previously, we have demonstrated the same drug loading effect using microcapsules of similar particle size distribution (Leelarasamee *et al.* 1986). Thus, there is no doubt that both the loading and the particle size distribution are key parameters for controlling the rate of drug release from the polylactic acid microcapsules. The present study shows that both of these parameters can be adequately controlled by the microencapsulation technique developed in this laboratory.

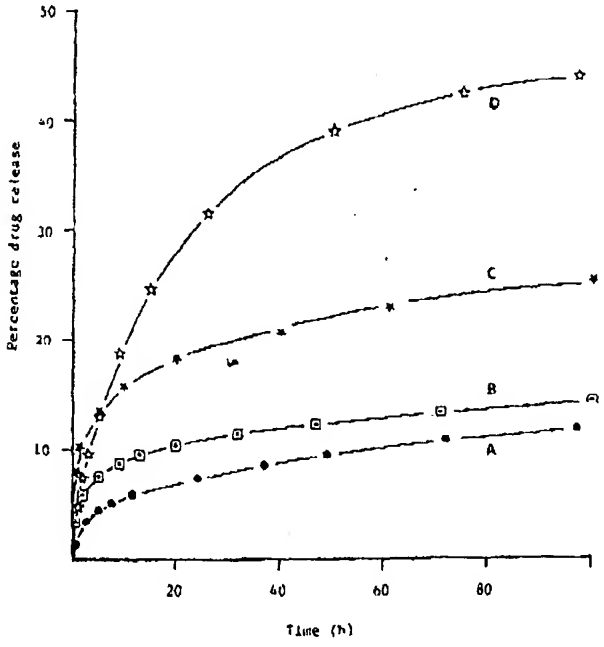
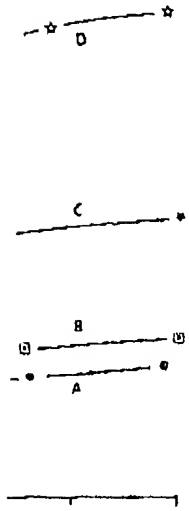


Figure 5. Drug release profiles for the hydrocortisone–polylactic acid microcapsules with an average diameter in the range of 200–223 μm and different drug loading: (A) 5·0 per cent; (B) 8·2 per cent; (C) 11·8 per cent; (D) 13·9 per cent.

: diffusional distance of the
g particles or clusters of drug
e. This may be especially true
ocapsule system, the particle
a change in particle size may

·cortisone from the polylactic
·lecule through the polymeric
·ene-vinyl acetate copolymer
of the macromolecules have
(Siegel and Langer 1984).
from microcapsules of similar
The results show that the rate
drug loading. Previously, we
·ing microcapsules of similar
Thus, there is no doubt that
key parameters for controlling
·ocapsules. The present study
·ly controlled by the microen-



·polylactic acid microcapsules with
d different drug loading: (A) 5·0 per
per cent.

References

Beck, L. R., Pope, V. Z., Flowers, C. E., Cowsar, D. R., Tice, T. R., Lewis, D. H., Dunn, R. L., Moore, A. B., and Gilley, R. M., 1983, Poly (DL-lactide-coglycolide) norethisterone microcapsules. An injectable biodegradable contraceptive. *Biology of Reproduction*, **28**, 186–195.

Benita, S., Benoit, J. P., Puisieux, F., and Thies, C., 1984, Characterization of drug-loaded poly (d,l-lactide) microspheres. *Journal of Pharmaceutical Sciences*, **73**, 1721–1724.

Benoit, J. P., Courteille, F., and Thies, C., 1986, A physicochemical study of the morphology of progesterone-loaded poly (D,L-lactide) microspheres. *International Journal of Pharmaceutics*, **29**, 95–102.

Cavalier, M., Benoit, J. P., and Thies, C., 1986, The formation and characterization of hydrocortisone-loaded poly (D,L-lactide) microspheres. *Journal of Pharmacy and Pharmacology*, **38**, 249–253.

Chang, R., Price, J. C., and Whitworth, C. W., 1986, Control of drug release rates through the use of mixtures of polycaprolactone and cellulose propionate polymers. *Pharmaceutical Technology*, **10**, 24–33.

Crank, J., 1956, *The Mathematics of Diffusion* (Oxford: Oxford University Press).

Cumming, J., and Willmott, N., 1985, Adriamycin loaded albumin microspheres: Qualitative assessment of drug incorporation and *in vitro* release by high performance liquid chromatography and high speed multi-diode array spectrophotometric detection. *Journal of Chromatography*, **343**, 208–212.

Fujimoto, S., Miyazaki, M., Endoh, R., Takahashi, O., Shrestra, R. D., Orui, K., Morimoto, Y., and Terao, K., 1985 a, Effects of intra-arterially-infused biodegradable microspheres containing mitomycin C. *Cancer*, **55**, 522–526.

Fujimoto, S., Miyazaki, M., Endoh, R., Takahashi, O., Orui, K., Sugibayashi, K., and Morimoto, Y., 1985 b, Mitomycin C carrying microspheres as a novel method of drug delivery. *Cancer Drug Delivery*, **2**, 173–181.

Higuchi, T., 1963, Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences*, **52**, 1145–1149.

Juni, K., Ogata, J., Matsui, N., Kubota, M., and Nakano, M., 1985, Control of release rate of bleomycin from polylactic acid microspheres by additives. *Chemical and Pharmaceutical Bulletin*, **33**, 1609–1614.

Leelarasamee, N., Howard, S. A., Malanga, C. J., Luzzi, L. A., Hogan, T. F., Kandzari, S. J., and Ma, J. K. H., 1986, Kinetics of drug release from polylactic acid-hydrocortisone microcapsules. *Journal of Microencapsulation*, **3**, 171–179.

Makino, K., Oshima, H., and Kondo, T., 1986, Mechanism of hydrolytic degradation of poly(L-lactide) microcapsules: Effects of pH, ionic strength and buffer concentration. *Journal of Microencapsulation*, **3**, 203–212.

Siegel, R. A., and Langer, R., 1984, Controlled release of polypeptides and other macromolecules. Pharmaceutical Research, *Journal of the Pharmaceutical-Biomedical Sciences*, **1**, 2–10.

Spenlehauer, G., Veillard, M., and Benoit, J. P., 1986, Formation and characterization of cisplatin loaded poly (D,L-lactide) microsphere for chemoembolization. *Journal of Pharmaceutical Sciences*, **75**, 750–755.

Sugibayashi, K., Akimoto, M., Morimoto, Y., Nadai, T., and Kato, Y., 1979, Drug carrier properties of albumin microspheres in chemotherapy III: Effects of microsphere entrapped 5-fluorouracil on Ehrlich ascites carcinoma in mice. *Journal of Pharmacobio-Dynamics*, **2**, 350–355.

Suzuki, K., and Price, J. C., 1985, Microencapsulation and dissolution properties of a neuroleptic in a biodegradable polymer, poly (D,L-lactide). *Journal of Pharmaceutical Sciences*, **74**, 21–24.

Tsai, D. C., Howard, S. A., Hogan, T. F., Malanga, C. J., and Ma, J. K. H., 1986, Preparation and *in vitro* evaluation of polylactic acid-mitomycin C microcapsules. *Journal of Microencapsulation*, **3**, 181–193.

Ziyan, H., and Ruiqui, X., 1985, A follow-up study of the efficacy and safety of injectable microencapsulated megestrol acetate and a discussion on its contraceptive mechanism. *International Journal of Gynaecology and Obstetrics*, **23**, 207–211.